

Assessment of Cardiac Oxidative Status of Diabetic Wistar Rats Exposed to Methanol Fraction of Ethanol Extract of *Dialium Guineense* Stem Bark

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ABSTRACT

The present study investigated the cardiac oxidative status of diabetic Wistar rats exposed to methanol fraction of ethanol extract of *Dialium guineense* (MEDG) stem bark. Male Wistar albino rats (n = 25, mean weight = 215 ± 15 g) were randomly assigned to five groups of 5 rats each: normal control, diabetic control, metformin, MEDG (200 mg/kg body weight, bwt) and MEDG (300 mg/kg bwt) groups. A single intraperitoneal injection of 50 mg/kg bwt STZ was used to induce diabetes mellitus in the rats. The diabetic rats were treated for 21 days with either metformin (50 mg/kg bwt) or MEDG stem bark. Activities of antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) as well as molecules like glutathione (GSH), total protein (TP), malondialdehyde (MDA) and nitric oxide (NO) were measured in heart homogenate (20 %). The results showed that induction of diabetes mellitus with STZ significantly increased the fasting blood glucose (FBG) concentrations of the rats, while decreasing the activity/concentration of antioxidant enzymes/molecules (p < 0.05). However, treatment of the diabetic Wistar albino rats with MEDG stem bark markedly reduced the FBG concentration and body weights of rats but enhanced the activity/concentration of antioxidant enzymes/molecules in cardiac tissue (p < 0.05). These results indicate that MEDG has the potential to promote antioxidant defense in the heart of STZ-induced diabetic rats.

Keywords: Antioxidants; Cardiac Tissue; Glutathione; Lipid Peroxidation; Nitric Oxide; Oxidative Stress

Introduction

As a meshwork of cardiac muscle cells interconnected by contiguous cytoplasmic bridges the heart pumps blood through blood vessels of the circulatory system [1,2]. Chemical-induced cardiac injury constitutes serious health risk to humans [3-5]. Besides its hepatotoxic effect STZ can negatively impact the heart [4]. Defined as the toxicity that affects the heart, cardiotoxicity is of special interest to researchers in the field of medicine [6]. It continues to top safety concerns principally because of lack of sufficient knowledge of the underlying mechanisms [7]. Cardiotoxicity is characterized by abnormality of cardiac electrical activity and contractile dysfunction, ultimately lead-

ing to heart failure. Cardiovascular adverse effects can lead to cardiac arrhythmias [8,9]. Oxidative and nitrative stress, and formation of protein adduct are established mechanisms of chemical-induced cardiotoxicity. Protein adduct formation causes cardiac muscle cell inflammation, perturbation of calcium homeostasis, apoptosis (programmed cell death), cardiomyocyte swelling, nuclear splitting, vacuolization, and alteration in signalling pathways [10]. Reactive oxygen species (ROS) produced as a result of oxidative stress cause damage to cellular structures within vascular wall, thereby triggering several redox-sensitive transcriptional pathways, shifting the cell towards a pro-atherogenic transcriptomic profile [11].

Diabetes mellitus is a disease characterized by chronic hyperglycemia [12-15]. A number of diabetics frequently combine oral antidiabetic medications with herbal supplements. In Africa, there are more than 500 plants that are known to have medicinal properties [16]. *Dialium guineense* (Velvet Tamarind) is a medicinal plant used in folklore medicine for the treatment of infections (diarrhea, severe cough, bronchitis, wound, stomachaches, malaria fever, jaundice, ulcer and hemorrhoids) [17]. It is a tall, tropical, fruit-bearing tree, belonging to the Leguminosae family, and has small, typically grape-sized edible fruits with brown hard inedible shells. In Africa, it grows in dense forests along the southern edge of the Sahel. The plant grows naturally in West African countries, Central African Republic, and Sudan [18]. In Nigeria, it is known by different names: Icheku (Igbo), Awin (Yoruba), Tsamiyarkurm (Hausa) and Amughen (Bini) [19, 20]. The aim of this study was to investigate the cardiac oxidative status of diabetic Wistar rats exposed to MEDG stem bark.

Materials and Methods

Drugs and Chemicals

The standard antidiabetic drug, metformin, was purchased from Micronova Laboratories (India), and STZ was a product of British Drug House (BDH) Chemicals Ltd. (England). Absolute ethanol, chloroform and other solvents were obtained from Bell, Sons & Co. (England), while formaldehyde was purchased from Thermo Fisher Scientific Ltd. (USA). All the chemicals and solvents used in this study were of analytical grade.

Collection of Plant Material

The authenticity of the stem barks of *D. guineense*, which were obtained from Auchi, Edo State, Nigeria, was verified by Dr. Henry Akinnibosun of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The prepared plant specimen was deposited in the herbarium of same department (No. UBHD330).

Plant Extraction

The plant's stem bark was washed and shade-dried for 2 weeks at room temperature, and thereafter ground into powder using a blender. A portion (500 g) of powdered plant material was steeped in 5,000 mL of 100 % ethanol. The resulting extract was filtered through muslin cloth and freeze-dried with a lyophilizer. The ethanol extract was subsequently fractionated with absolute methanol [21,22].

Animals

Male Wistar albino rats (n = 25, mean weight = 215 ± 15 g) were bought from the Department of Anatomy, University of Benin, Nigeria and housed in wooden cages. They were acclimatized for fourteen days before commencement of the study and had free access to feed and water.

Experimental Design

The rats were randomly assigned to five groups (5 rats per group): normal control, diabetic control, metformin, MEDG (200 mg/kg bwt) and MEDG (300 mg/kg bwt) groups. Diabetes mellitus was induced in the rats via intraperitoneal injection of STZ at a dose of 50 mg/kg bwt. The diabetic rats were then treated with either metformin (50 mg/kg bwt) or the extract, for 21 days.

Tissue Sample Collection and Preparation

At the end of day 21 of treatment, the rats were euthanized under mild chloroform anaesthesia after an overnight fast. Their hearts were excised and used to prepare 20 % tissue homogenate. The homogenate was centrifuged at 2000 rpm for 10 min to obtain clear supernatant.

Biochemical Analyses

The activities of catalase, SOD and GPx were determined [23-25]. Cardiac levels of TP, MDA, GSH, and NO were also measured [26-29]. The activity of GR was determined using a previously described method [30].

Data Analysis

Data are presented as mean ± SEM (n = 5). Statistical analysis was performed using SPSS version 21. Statistical differences between means were compared using Duncan multiple range test. Statistical significance was assumed at p < 0.05.

Results

Effect of MEDG Stem Bark on Weight and Blood Glucose of Rats

Induction of diabetes mellitus with STZ significantly increased the blood glucose concentrations of the rats (p < 0.05). However, treatment of the diabetic rats with MEDG stem bark markedly reduced the FBG concentration and body weights of rats (p < 0.05) (Table 1 and Figure 1). Data are weight and FBG parameters and are expressed as mean ± SEM (n = 5).

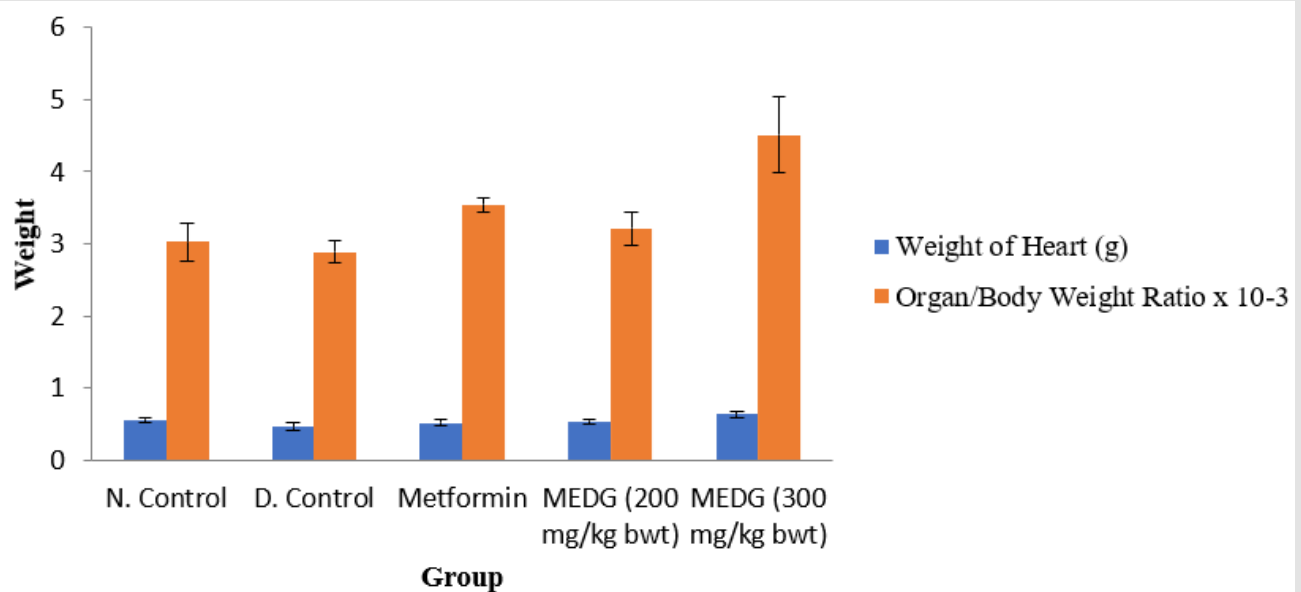


Figure 1: Comparison of Organ and Relative Organ Weights.

Table 1: Weight and Blood Glucose Parameters.

Group	Weight Change (g)	Weight Change (%)	FBG (mg/dL)	Glycemic Change (mg/dL)	Glycemic Change (%)
Normal Control	-	-	-	-	-
Diabetic Control	-	-	> 800	-	-
Metformin	20.35	12.16	> 800	399	49.88
MEDG (200 mg/kg bwt)	16	11.19	427	311	71.61
MEDG (300 mg/kg bwt)	27	15.68	467.67	394.33	78.19

Cardiac Oxidative Status of Diabetic Rats

Induction of diabetes mellitus with STZ markedly reduced the activities of the markers of oxidative stress, % GSH, and % NO in cardiac

tissue ($p < 0.05$). However, treatment of diabetic Wistar albino rats with MEDG stem bark significantly increased the activities of antioxidant enzymes and other parameters measured ($p < 0.05$; Figures 2-4).

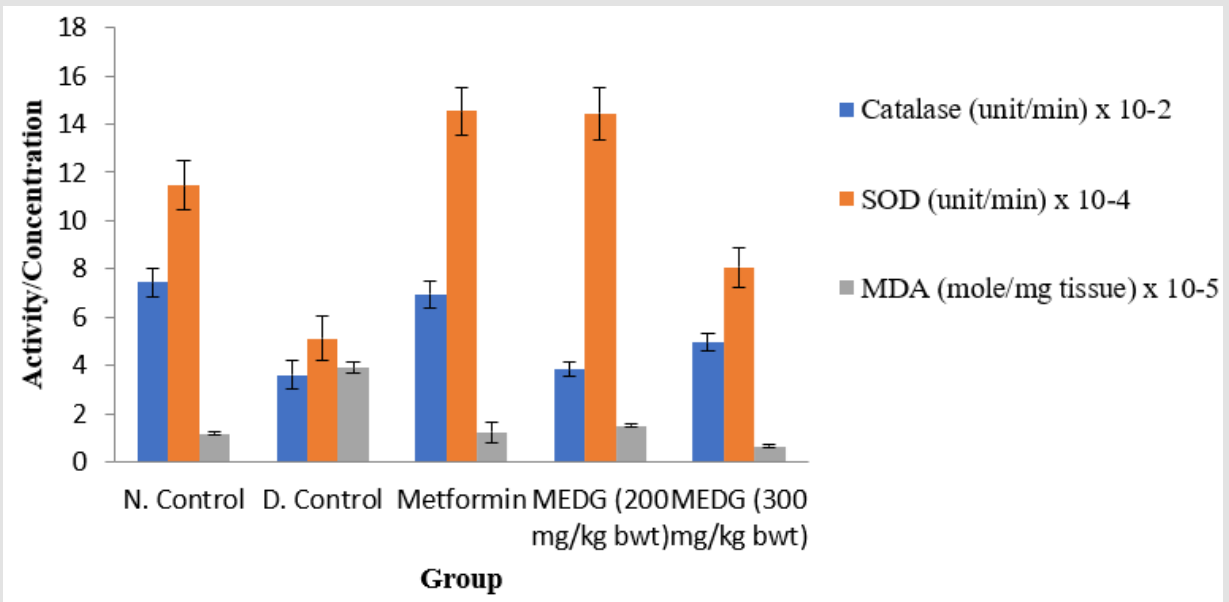


Figure 2: Effect of MEDG Stem Bark on Oxidative Status in Cardiac Tissue.

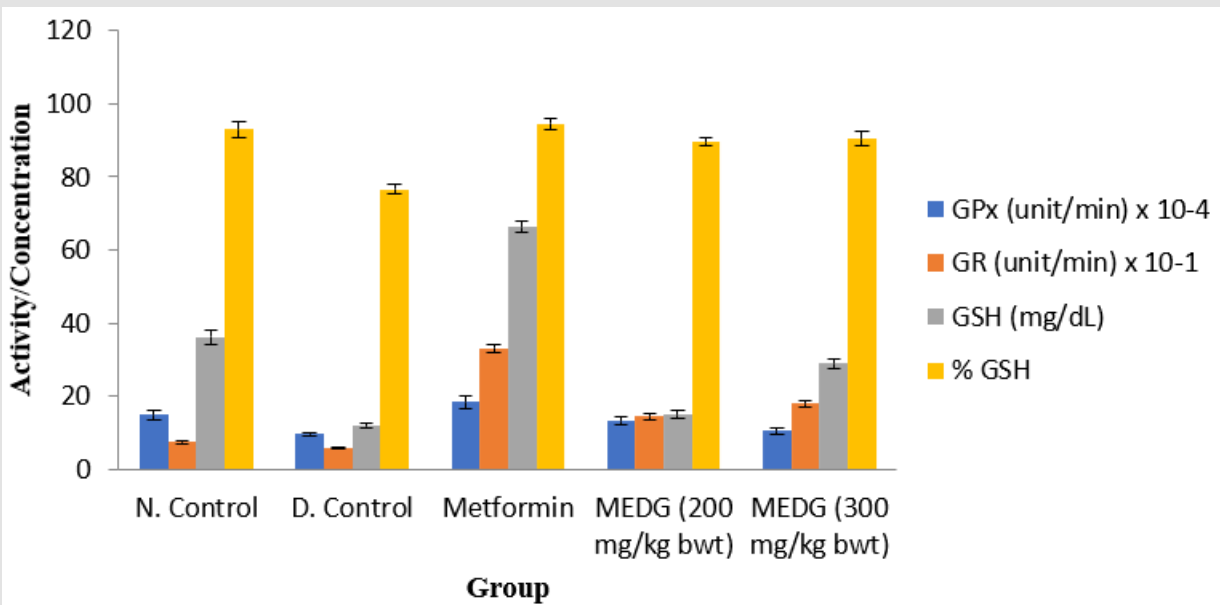


Figure 3: Effect of MEDG Stem Bark on Glutathione Enzyme System.

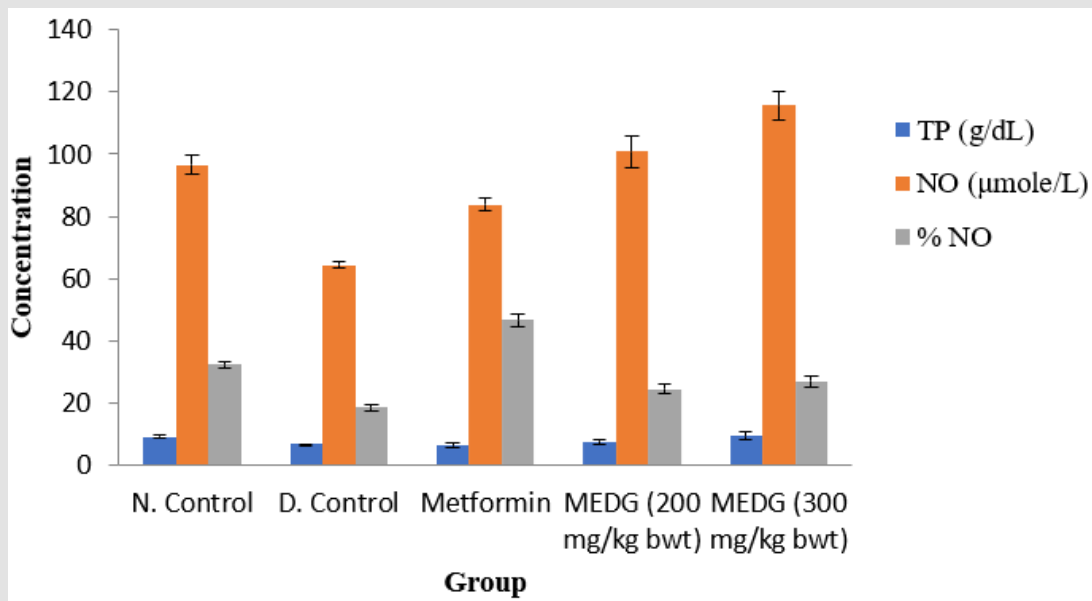


Figure 4: Effect of MEDG Stem Bark on cardiac TP and NO Levels.

Discussion

Diabetes mellitus refers to a group of metabolic disorders characterized by a state of chronic hyperglycemia due to defects in insulin secretion, insulin action or both [31]. Over time, elevated blood glucose leads to serious damage to the heart, blood vessels, eyes, kidneys and nerves. This metabolic disorder is often described as “a silent killer” since it may be asymptomatic at onset. Therefore, the disease usually goes undiagnosed until major complications arise. Diabetes mellitus is associated with reduced life expectancy, significant morbidity as well as diminished quality of life [32-34]. It has been reported that 10 % of global health expenditure is spent on the disease annually [35,36]. There is profound reason to suggest that this figure might increase in the coming years giving the myriads of complications that result from diabetes mellitus. Oxidative stress has a mediatory role in the pathogenesis of diabetes mellitus and its related complications via promotion of free radicals production and impairment of antioxidant defense systems [36]. This study investigated the cardiac oxidative status of diabetic Wistar rats exposed to MEDG stem bark. In this study, albino rats of Wistar strain were used, since they are a well-known animal model. The results showed that diabetic rats’ heart measurements were much worse than those of the normal control, which suggests that diabetic cardiomyopathy was present.

Also, the loss of aorta’s ability to relax when NO is present shows that endothelial dysfunction also occurred. Compared to normal control rats, there were also more signs of oxidative stress, and inflammation. These symptoms of diabetes mellitus are well-known and are considered to be some of the main causes of the major complications. Diabetes mellitus tends to damage cell membrane, thereby

enhancing the production of ROS [12,13]. Studies have demonstrated that diabetic patients experience increased oxidative and nitrosative stress, which can negatively affect the heart or arteries. In this study, induction of diabetes mellitus with STZ markedly reduced the activities of the markers of oxidative stress, % GSH, and % NO in cardiac tissue. However, treatment of diabetic Wistar rats with MEDG stem bark significantly increased the activities of antioxidant enzymes and other parameters measured. It is likely that in the myocardium STZ aggravated oxidative stress process by suppressing the expression of SOD, catalase, and GPx.

These results are consistent with those of previous studies [37-46]. The results suggest that STZ-induced diabetes mellitus promotes increased production of ROS, while suppressing the synthesis/activity of antioxidant enzymes/molecules in myocardial tissue. The MEDG stem bark may contain compounds that exert anti-hyperglycemic effects as well as oxidative stress-reducing properties in STZ-induced diabetic rats. Containing important phytochemicals, the medicinal plant has been demonstrated to exhibit a number of biological/pharmacological effects [47-83].

Conclusion

According to the findings of this study, methanol fraction of the ethanol extract of *Dialium guineense* stem bark is able to alter the oxidative status of the hearts of diabetic rats. The findings have given credence to the use of *Dialium guineense* in Traditional Medicine for the treatment of diabetes mellitus. However, additional work is required to ascertain its mode of action and determine whether or not it is harmful in the long-term.

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